found on page 9, lines 9-10. Support for claim 80 can be found on page 9, lines 4-5, and example 4. Support for claim 81 can be found on page 8, lines 11-13. Support for claim 82 can be found on page 8, line 12, and example 5. Claims 83 and 84 are supported by lines 18-22 of page 8 of the specification. Claim 85 is supported by page 8, lines 11-13 of the specification. Support for claim 86 can be found on page 9, lines 4-5. Support for claim 87 can be found in example 4.

Support for claim 88 can be found on page 9, lines 10 and 18-21. Support for claims 89, 90 and 91 can be found on page 9, lines 24-27. Support for claim 92 can be found in examples 4 and 5. Support for claim 93 can be found on page 5, line 33-page 6, line 2 and page 9, lines 18-27. Support for claim 94 can be found in example 4. Further support for the claims can be found throughout the specification and the claims.

The amendment to the specification is made to correct an obvious typographical error. As is apparent from the following sentences in the paragraph on page 6, vegetable oils contain polyunsaturated fatty acids (PUFAs) but do not comprise the long chain PUFAs (PUFAs of 20 carbons or more).

In the Office Action, the examiner rejected claims 1-66 under 35 U.S.C. § 103 as unpatentable over Japanese Patent Application 196,255 (Suntory) and PCT Application WO89/00606 (Long) in view of Clandinin et al. and Traitler et al. The examiner noted that the Suntory application teaches the process of adding lipid material produced by *Mortierella* to supplement

milk for infants. The lipids were said to contain polyunsaturated fatty acids such as GLA, ARA and EPA. The Long application was cited as teaching supplementing infant formula with lipids containing the omega-3 polyunsaturated fatty acids DHA and EPA. The examiner stated that the lipids were obtained from various sources, including *Pythium* and *Crypthecodinium*. The examiner further stated that the subject matter of claims 1-66 differed from that of the primary references in claiming the addition of GLA obtained from plant and fish oils. She further averred that it would have been obvious to supplement infant formula with GLA from such sources given the teachings of Clandinin and Traitler. Clandinin was cited as teaching the addition of fatty acids from fish oil to infant formula. Traitler was cited as teaching the addition of GLA to infant formula wherein the GLA was obtained from black currant oil. The examiner asserted that the choice of ratios of ingredients was within the skill of one skilled in the art. To the extent that this rejection could be applied to new claims 67-94, this rejection is traversed.

The invention of the claims of this application is directed to blends of oils comprising certain long chain polyunsaturated fatty acids. More specifically, as set forth in the claims, the invention is directed to blends of oils enriched in ARA and in DHA, wherein the resultant oil blend is at least substantially free of EPA, and the DHA and ARA, as well as any small amounts of EPA present, are in the form of triglycerides. Specifically, the

ratio of ARA:EPA in the oil blend is from about 20:1 to about 5:1, and in some instances, the oil blend may be essentially free of EPA. The claims also are directed to a process for supplementing infant formula by adding blends of oils comprising these fatty acids and to infant formula comprising these oil blends. The infant formulas obtained comprise ARA and DHA in amounts comparable to the amounts present in human breast milk. The infant formula also is characterized by having an amount of EPA comparable to the amount of EPA in human breast milk.

EPA is a desirable fatty acid component of certain products. Over the last several years, however, there has been increasing evidence accumulating that relatively high amounts of EPA are not desirable components of other products, particularly infant formula. Studies have shown that high levels of EPA in infant formula can be deleterious to infant development. See, for example, Carlson, S.E., et al., *Essential Fatty Acids and Eicosenoids*, page 192, A. Sinclair and R. Gibson, eds. (1992) (a copy of which is enclosed) who recommended that infant formula be supplemented with DHA alone, rather than a combination of DHA and EPA, in view of their findings that the administration of formula comprising significant amounts of EPA resulted in negative effects on infant growth and psychomotor development.

The fatty acid composition of human milk is shown in Table 6 (page 136) of *Omega-3 Fatty Acids in Health and Disease*, Robert Lees and Marcus Karel, eds., (1990). A copy of this table is enclosed. As shown therein, EPA generally comprises only about

0.03% of the fatty acid composition. ARA, in contrast, comprises about 0.59%, and DHA comprises about 0.19%, of the fatty acid composition of human breast milk[1]. These fatty acids, as well as the others present in human breast milk, are provided in the form of triglycerides. Manufacturers of commercial infant formula have tried to duplicate the fatty acid composition of breast milk, but have been unable to do so. Consequently, no commercially available formulas to date contain DHA or ARA in triglyceride form. Prior to Applicant's invention, there were no sources for DHA and ARA that would provide commercially significant quantities of these desirable fatty acids in triglyceride form without the concomitant production of significant amounts of EPA.

Applicant has found that both DHA and ARA can be produced by microbial sources and that these sources can produce triglyceride oils enriched in the fatty acid of interest and at least substantially free of EPA. Applicant has demonstrated how these oils can be blended to provide DHA and ARA in desired relative amounts and used to supplement nutritional products, including infant formula. The resultant infant formulas, for example, comprise ARA, DHA and EPA in amounts comparable to the corresponding amounts in human breast milk. Another advantage of the Applicant's invention is economic; because the oils are so

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[1] These percentages represent average levels. In individual instances, the amounts of one or more of these fatty acids may vary. See, for example, Table 1 of the Clandinin et al. reference cited by the examiner.

highly enriched in the fatty acids of interest, only relatively small amounts of the oils need be added to the infant formula. As a result of Applicant's work, one now can very precisely obtain, in an economically viable process, a composition comprising desired absolute and relative amounts of DHA and ARA in triglyceride form that is free of undesired amounts of another long chain polyunsaturated fatty acid (PUFA), EPA.

Similarly, claims 88-91 set forth above are directed to compositions providing a combination of a microbially-produced oil comprising DHA and an oil comprising GLA. As discussed in the specification, GLA is a precursor of ARA and, in certain circumstances, it may be desirable to provide the precursor, rather than ARA itself, in a composition. Again, a distinguishing feature of the claimed compositions is that it provides the two fatty acids of interest in triglyceride form and in an environment that is free of unwanted EPA.

The references cited by the examiner do not teach or suggest triglyceride oil compositions which comprise DHA and either ARA or GLA and are substantially free of EPA. More specifically, although the two primary references were cited by the examiner as teaching the components of Applicant's oil mixtures, neither of these references discloses or suggests obtaining or using oils having the key characteristics of the oil components of the Applicant's invention. In each of the two primary references it is deemed acceptable, and even desirable, to supplement infant formula with lipids containing significant amounts of EPA, and

neither reference discloses the use of oils enriched in DHA and ARA but deficient in EPA as a nutritional supplement. The deficiencies of the primary references are not compensated by the secondary references cited in the Office Action.

The Suntory application is directed to the addition of one or more fatty aids to infant formula. The application teaches the addition of the fatty acids gamma linolenic acid (GLA), bis-homo-gamma-linolenic acid (DGLA), arachidonic acid (ARA), eicosadienoic acid (EDA) and eicosapentaenoic acid (EPA), either alone or in combination, to manufactured milk. As an initial point, it should be noted that the application makes no reference to DHA or oils comprising DHA. Thus, the teachings of this reference are relevant, if at all, only to the ARA- or GLA-containing component of the compositions of the present invention.

Suntory discloses that the fatty acids can be in the form of free fatty acid, fatty acid esters, oils and fats, hydrolysates of the oils and fats, or esterified products of dissolved matter of the fats and oils. The Suntory application provides that these additives can be produced by yeast methods or fermentation methods, but the application provides very little information as to sources of the fatty acids. Although the application contains examples (see examples 1 and 2) in which a single fatty acid is added to milk, there is no discussion as to the source used to produce the fatty acid, how it was obtained to the exclusion of, or isolated from, other fatty acids, or how to obtain it in the

form of a triglyceride. The only microorganisms mentioned in the application are *Mortierella* organisms, but in the examples in which a *Mortierella* strain was cultivated to produce fatty acid, the product obtained was a mixture of fatty acids that contained ARA and significant amounts of EPA, as well as other PUFAs. For instance, in example 4 of the application, *Mortierella aeromonas* was cultured to produce an "oil and fat" that was extracted from the organism and converted to produce an ethyl ester product. The composition of the product was found to contain the ethyl esters of palmitic acid, stearic acid, oleic acid, linolenic acid, GLA, DGLA, EDA, ARA and EPA. The example states that the amount of EPA present in the mixture is 50% of the amount of ARA. This composition containing the mixture of ethyl esters was added to powdered milk. Clearly, this is distinguishable from the Applicant's invention.

Example 6 teaches adding the ethyl esters of GLA, EDA, DGLA, ARA and EPA to cyclodextrin in relative weight percentages of 2:1:6:4:8 and adding the resultant product to powdered and liquid milk. In this example, in which specific relative amounts of each of the fatty acids of interest are provided, *twice as much* EPA as ARA is added to milk. The use of such mixtures to supplement infant formula is fundamentally different from the invention claimed by the Applicant.

Furthermore, although the Suntory application provides that the fatty acids or their salts or esters can be utilized without further modification, it teaches that it is a "good idea" to

first encapsulate the substance in cyclodextrin. In examples 1 and 2 of the application, the applicants teach adding 1 gram of cyclodextrin powder containing 5 % of one of the fatty acids of interest to 1 kg of a powdered milk. This is the equivalent of adding 50 mg of fatty acid to 1 kg of milk powder. If the fatty acid is ARA (example 2), this amount would be much less than the amount necessary to match the amount found in human breast milk. Typically, there are 250 g fat per kg of milk powder (see, for example, Similac™, Ross Laboratories). Human breast milk comprises about 0.5-0.7 % ARA, as cited above. Thus, to provide a milk powder having an amount of ARA comparable to that in human breast milk, one would need to add about 1.3-1.8 g ARA per kg of the powdered milk. Suntory's example thus teaches only adding about three one-hundredths (3%) of the desired amount of ARA. In order to provide a milk product having an amount of ARA comparable to the amount found in human breast milk, approximately 30-40 g of the Suntory ARA-containing cyclodextrin would have to be added to 1 kg of powdered milk. Such an amount of an artificial additive like cyclodextrin certainly would not be desirable in infant formula, where the goal has always been to provide a formula as close to natural, human breast milk as possible. In contrast to the Applicant's invention, the Suntory application does not teach or suggest how to obtain an infant formula comprising ARA in an amount comparable to the amount of ARA found in human breast milk.

Furthermore, this reference is deficient in that it does not teach or suggest adding to infant formula a microbial triglyceride oil enriched in ARA (or GLA) but substantially free of EPA. Although the production of lipids comprising ARA and low amounts of EPA by *Mortierella alpina* is known in the art, see Shinmen et al., *Appl. Microbiol. Biotechnol.* 31:11 (1989), a copy of which is enclosed, there is no suggestion in the art of adding to infant formula a microbial triglyceride oil enriched in ARA and substantially free of EPA. Indeed, the Suntory application teaches away from this by specifically providing that mixtures of fatty acids which include EPA in relatively high amounts can be added to milk. As noted above, in at least one example of the Suntory application, the applicants advocate adding twice as much EPA as ARA to milk. In contrast, the present specification and claims provide for adding ARA and EPA in amounts comparable to the amounts in human breast milk. In addition, Applicant teaches and claims supplementing infant formula with oils which are enriched in ARA and DHA and are essentially free of EPA. Such an embodiment is highly desirable in view of the warnings raised in the literature today regarding the problems associated with providing EPA in infant formula.

In addition, the Suntory application clearly teaches that the fatty acids preferably are added to milk only after they have been encapsulated in cyclodextrin. In this form, it clearly does not match the form of ARA in human milk. Thus this reference does not disclose or suggest a means of supplementing infant

formula by adding ARA, DHA and EPA in amounts comparable to the amounts of, and in the same form as, the ARA, DHA and EPA in human breast milk.

The second reference cited by the examiner, the PCT application by Long, discusses culturing a variety of microorganisms to produce omega-3 fatty acids (for example, DHA and EPA) that then can be used in food, cosmetic and pharmaceutical products. This application contains only prophetic examples. In each of examples 1-5, Long provides general procedures for culturing any of a variety of microorganisms, including fungi and microalgae, and then harvesting the cells and recovering the lipids produced. At the end of the examples, Long states that the lipid fractions contain omega-3 fatty acids, which then are esterified to produce the corresponding methyl esters. He goes on to provide that the omega-3 fatty acids

> may constitute as much as 10 to 50% of the total fatty acid fraction. They are generally contained in phospholipids, glycolipids, mono-, di-, or triglycerides, *and* sulpholipids, or as the free acids, but are not limited to these forms.

(page 8, lines 7-10 [Emphasis added]).

Long does not teach or suggest obtaining a triglyceride enriched in a single omega-3 fatty acid, such as DHA, much less obtaining such a triglyceride without the concomitant production of significant amounts of EPA, another omega-3 fatty acid. Applicants are claiming oil compositions in which one component

is an oil enriched in DHA, and a key feature of the oil composition is that it is substantially free of EPA. Long teaches only how to obtain a variety of omega-3 fatty acids in a complex and unseparated form. Anyone reading the passage quoted above from the Long application would believe that one could not obtain a single omega-3 fatty acid, such as DHA, by cultivating a microorganism.

Additionally, as also is apparent from the Long passage quoted above, Long provides that from 10-50% of the total fatty acid fraction produced may constitute omega-3 fatty acids. The total amount of DHA, one particular such fatty acid, in one particular form of lipid (triglyceride), therefore is likely to be quite small. Thus, from Long's disclosure, one knows only that any of a list of eukaryotic microorganisms can be cultivated and will be found to contain a variety of omega-3 fatty acids and that these fatty acids can be present in a complex, unrecoverable form. There is absolutely no teaching in the Long application that an oil enriched in a single desired fatty acid, DHA, can be obtained from a microbial source, recovered and then used with an oil enriched in ARA or GLA to provide a nutritional supplement, such as a supplement for infant formula.

To illustrate that the procedures set forth in the Long application do not teach one how to obtain a DHA-enriched triglyceride oil, Applicant has faithfully and precisely carried out the specific prophetic examples in the Long application, culturing a number of different strains of *C. cohnii* using

culture media comprising various concentrations of carbon and nitrogen in accordance with Long's teachings. He was not able to obtain in any of these experiments a single cell oil enriched in DHA, wherein the DHA is provided in the form of a triglyceride (i.e., the form used by the Applicant in the processes and compositions of his claimed invention). The results of these experiments are provided in the Declaration Pursuant to 37 C.F.R. § 1.132 provided herewith. As can be seen from the declaration, time after time, the culturing produced zero to very low yields of DHA. At no time did the Applicant obtain a recoverable oil comprising at least 25% DHA.

The secondary references do not cure the deficiencies of the primary references. Clandinin et al. teach the use of egg yolk lipid and fish oil to provide an edible fat composition for incorporation into an infant formula. Nowhere do Clandinin et al. recognize, teach or suggest that microbial oils can provide the fatty acids useful for supplementing nutritional products such as infant formula.

Furthermore, Clandinin uses fish oil as a source of the omega-3 fatty acids to be added to infant formula. Fish oil comprises DHA, but it also generally comprises significant amounts of EPA. If fish oil is added to an infant formula in a sufficient amount to provide an amount of DHA comparable to that found in human breast milk, the level of EPA in the formula typically will be much higher than the level of EPA in breast milk. Table 7 of the Clandinin patent provides the fatty acid

composition of infant formula made with fish oil. The amount of ARA is only about one quarter of the amount found in human breast milk (0.13% rather than 0.6%), yet the amount of EPA is more than 60 times higher than the amount in breast milk (2.03% vs. 0.03%)[2]. Clandinin's formula also has no detectable amounts of DHA. As has been noted above, recent scientific evidence indicates that the administration of high levels of EPA to infants can be deleterious to their development. Specifically, for example, it has been shown that fish oil generally is not suitable for infant formula supplementation as the EPA inhibits the conversion of linolenic acid to ARA in the infant's body. See Carlson, S.E. et al., referenced above. See also, Bjerve, K.S., et al., *Am. J. Clin. Nutri. 57*(supp): 801S (1993), a copy of which is enclosed. Thus, although fish oil provides one desirable fatty acid, DHA, it can hinder structural and organ development by limiting availability of a second important fatty acid, ARA.

Clandinin also discloses the use of egg yolk lipid as a source for omega-6 fatty acids. Approximately 2% of the total lipid in egg yolk lipid is ARA; little EPA or DHA is found in egg yolk lipid. The long chain fatty acids in egg yolk lipid, however, are in the form of phospholipids. In contrast to this, the oils of the Applicant's claims provide the fatty acids in the

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[2] Even if one uses Clandinin's own calculations for the fatty acid composition of breast milk, the amount of EPA in the fish oil-containing infant formula of Table 7 still is about 17 times higher than the amount of EPA in breast milk (2.03% vs. 0.12%).

form of neutral triglycerides. It is known that phospholipids, such as egg yolk lipids, are digested and metabolized differently than are triglycerides. More specifically, the enzymes which remove fatty acids from neutral triglycerides are different from those which metabolize polar phospholipids. Lingual and pancreatic lipases act on the triglycerides, whereas phospholipases act on phospholipids. As most, and perhaps all, of the omega-3 and omega-6 fatty acids present in human milk is present in milk fat globules, i.e., neutral triglyceride form, the ARA and DHA provided in the compositions of the Applicant's invention are in the same form as those found in human breast milk. Clandinin does not teach or suggest how to achieve a composition which provides key fatty acids in the same form and in the same amounts as found in human breast milk and which does not contain amounts of a fatty acid (EPA) now believed to be deleterious to infant development greater than the trace amounts that exist in human breast milk.

The last reference cited by the examiner, Traitler et al., discloses only obtaining an oil comprising GLA from a fruit. This reference does not teach or suggest combining such oils with microbial oils enriched in DHA and substantially free of EPA.

To summarize, none of the cited references, when considered independently or in combination, disclose or suggest mixtures of triglyceride oils enriched in ARA and DHA, wherein the mixture is further characterized by being substantially free of EPA. In addition, the cited references do not teach or suggest mixtures

of oils comprising a triglyceride oil enriched in DHA and an oil comprising GLA, wherein a further characteristic of the mixture is that it does not comprise significant amounts of EPA. The cited references further do not teach how to obtain an infant formula comprising DHA, ARA and EPA in the same form as, and in amounts comparable to the amounts of, these fatty acids in human breast milk.

In view of the foregoing amendments and discussion, Applicant respectfully submits that the claims of the application are in condition for allowance.

Respectfully submitted,

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